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Another case of coincidental *Giardia* infection and pancreatic cancer

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ABSTRACT

Until now, few cases of coincidental giardiasis and pancreatic tumors have been described. Among these cases, three described giardiasis cases coincided with confirmed pancreatic cancer. We present another case of *Giardia* infection coexisting with pancreatic cancer in a 67-year-old man who suffered from stenosis of the distal ductus choledochus combined with a hypoechoic mass in the head of the pancreas. The diagnostic conclusion of suspicious adenocarcinoma was based on endoscopic ultrasound fine-needle aspiration (EUS-FNA) biopsy and confirmed by a partial duodenopancreatectomy. On bloody cytology smears prepared from the EUS-FNA specimen, trophozoites of *Giardia intestinalis* accompanying an inflammatory background and features that fulfilled the morphological criteria of malignancy were observed. In histological sections from the duodenopancreatectomy specimens, the parasites were observed attached to the epithelium, but individual *Giardia* parasites were also observed beneath the epithelial lining. According to conventional genotyping, the infecting *Giardia* belonged to sub-assemblage AII.

1. Introduction

Giardiasis is the most common waterborne parasitic infection present in both developing and developed countries around the world. In developed countries, the prevalence of giardiasis ranges from 3% to 7%. In developing countries, 20% to 100% of the population may be affected by this disease [1].

Giardiasis is an infection of the mucosal surface of the upper small intestine caused by the noninvasive parasitic flagellate *Giardia intestinalis* (syn. *G. duodenalis*), which is transmitted via environmental cysts. Approximately half of infected individuals remain asymptomatic. Most symptomatic cases manifest as uncomplicated diarrheal disease with foul-smelling, greasy stools and flatulence, but in some patients, particularly in small children in endemic resource-limited settings, chronic infection can lead to malabsorption syndrome and other pathologic sequelae [2,3]. In addition, the coincidence of giardiasis with other diseases, such as pernicious anemia, has been described [4]. Additionally, sporadic case reports on *Giardia* infection coexisting with tumor masses in the pancreas or gallbladder have been published [5–8] provoking speculation regarding the potential relationship between giardiasis and carcinogenesis [7,9,10]. Here, we present another case of originally unrecognized, likely chronic asymptomatic giardiasis coincident with pancreatic carcinoma. This case is also unusual because of the intra- and subepithelial localization of the parasites in the duodenum.

2. Case

A 67-year-old man with hypertension and chronic persistent hepatitis B was admitted to a district hospital with a 2-month history of icterus, unexplained significant weight loss, and elevated hepatic and extrahepatic laboratory parameters. The patient previously smoked 10 cigarettes per day. Upon admission, the patient complained of mild abdominal pain without diarrhea or vomiting. He underwent endoscopic retrograde cholangiopancreatography (ERCP) that showed stenosis of the intrapancreatic portion of the distal common bile duct (CBD) and a 2.8 cm (in the largest diameter) hypoechoic mass in the pancreatic head suspicious of malignancy. The patient then underwent a transduodenal endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsy. Diff-Quick-stained cytology smears prepared from specimens obtained by EUS-FNA biopsy showed three-dimensional clusters with overlapping nuclei or isolated lamellae of the cylindrical

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epithelium with nuclear atypia of various grades, which were suspicious of adenocarcinoma. An inflammatory background that fulfilled the morphological criteria of malignancy was observed near the clusters and the isolated elements (Fig. S1). Further, trophozoites of the parasite *G. intestinalis* were observed on the smears (Fig. 1a and Fig. S2). Based on the cytopathology findings, the patient underwent a partial duodenopancreatectomy. Histopathological examination of duodenopancreatectomy specimen sections indicated invasive ductal carcinoma showing well to moderately differentiated adenocarcinoma with extensive perineural and endolymphatic propagation (Fig. S3). When examining the duodenal mucosa, exaggerated chronic lymphocytic infiltration without activity was observed in the tissues, and many *Giardia* trophozoites attached to enterocyte microvilli were observed on the mucosal surface (Fig. 1b–d). Surprisingly, single parasites were also situated between neighboring enterocytes (Fig. 1d) or beneath the duodenal epithelium, where they were almost exclusively observed in the space formed under the epithelial lining detached from the basement membrane (Fig. 1b, c). Using a spin column-based QIAamp DNA Blood and Tissue Mini Kit (Qiagen), genomic DNA was isolated from a *Giardia*-positive, Diff-Quick-stained permanent cytology smear. Multilocus genotyping of *Giardia* was conducted via sequencing portions of three genes, namely, beta-giardin (*bg*), glutamate dehydrogenase (*gdh*) and triose phosphate isomerase (*tpi*), which are commonly used to differentiate genetic groups (called assemblages) of *G. intestinalis* [11]. Conventional genotyping revealed sub-assemblage AII as a causative agent of the patient's ongoing giardiasis. After *Giardia* was identified in biopsy specimens and before treatment, the patient's stool sample was examined for *Giardia* cysts with negative results. Despite chemotherapy, the patient succumbed to the cancer several months later.

3. Discussion

A few reports have described the coexistence of *Giardia* infection with tumors or cysts in the pancreas [5–7,10,12–14]. Three of the cases presented pathologically confirmed carcinomas [5–7], and the others described coincidental findings of *Giardia* and neoplasms in the pancreatic tissues without evidence of malignancy [10,12,13]. Thus, to the best of our knowledge, our case represents the fourth published case of coincidental pancreatic carcinoma and giardiasis. All patients in whom the coincidence of infection and pancreatic neoplasm has been found were adults between the ages of 53 and 74 years. In our case, and in those reported by Kurita et al. [6] and Furukawa et al. [7], giardiasis coincided with pancreatic ductal adenocarcinoma (PDAC) in patients between 59 and 69 years of age. PDACs are the twelfth most common tumors in the world, affecting predominantly older adults [16]. The reported prevalence of giardiasis is from 3% to 7% in high-income countries [1]. In the Czech Republic in the 59–69 age group, the incidence of PDAC is currently between 48.72 and 54.23 cases per 100,000 inhabitants [17]. In contrast, the incidence of giardiasis is significantly lower, between 0.75 and 1.32 cases per 100,000 inhabitants and year, and involves the whole age spectrum (according to the National Reference Laboratory for Diagnostics of Intestinal Parasitoses, Prague, the Czech Republic) [18]. The probability of the coincidence of both diseases therefore seems to be very low, and the very low number of coincidental cases described so far, including our case, does not support a relationship between giardiasis and carcinogenesis.

Giardiasis was not suspected in any of the aforementioned cases, including in our case, and the infection has been always recognized incidentally, usually by observing *Giardia* on stained cytology smears prepared from EUS-FNA specimens or from the pancreatic juice of a patient examined for a pancreatic mass. Contamination of FNA specimens with luminal content has been suspected as a source of *Giardia* on

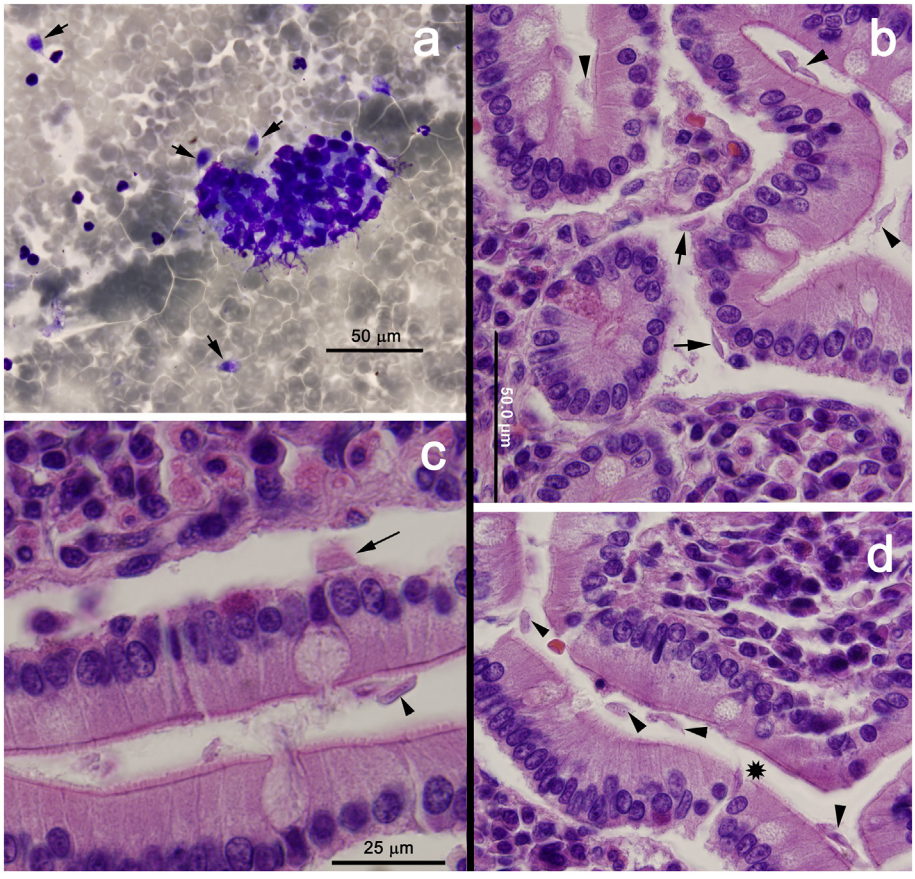

Fig. 1. Trophozoites of *G. intestinalis* in pathological specimens from the pancreas and the duodenum. (a) A Diff-Quick-stained cytology smear of bloody aspirate from EUS-FNA showing individual trophozoites of *Giardia* (arrows) dispersed among erythrocytes; a cluster of cells with large hyperchromatic nuclei representing reactive changes is visible in a center of the smear. (b–d). H&E-stained histological sections of the duodenum from duodenopancreatectomy demonstrating intraluminal *Giardia* trophozoites (arrowheads) adherent to or swimming near the surface of the duodenal epithelium. (b, c) Single trophozoites (arrows) are seen subepithelially. In (d), a *Giardia* trophozoite (asterisk) between epithelial cells can be observed. Magnification in (d) is the same as in (b).

smears prepared from duodenal aspirates by Mitchell et al. [5] and Carter et al. [13]. In our case, we could not completely exclude contamination of the specimens; however, contaminating epithelial cells were not observed on the smears. Epithelial cells, which usually form cohesive, uniform cell groups with a "starry sky" pattern of clear goblet cells [15], were also not reported by others [10,13] in contrast to the presence of atypia of various grades or atypical epithelial cells [10], [this paper].

Localization of *Giardia* between and below epithelial cells, as we observed on histology sections from the duodenum, is highly atypical because it is generally accepted that *Giardia* is a luminal parasite and that the trophozoites reversibly adhere to but do not invade the epithelium (reviewed in [3]). In our case, we cannot exclude accidental invasion following the collapse of epithelial barrier caused by cancer-related inflammatory reactions. On the other hand, there are several reports describing observations of *Giardia* parasites within the duodenal mucosa during giardiasis not connected to any inflammatory disease [19–22]. In our case, as well as in all of the abovementioned cases, numerous *Giardia* resided intraluminally, but only single parasites were typically observed intraepithelially and subepithelially in the lamina propria. When comparing giardiasis cases in which extraluminal *Giardia* was observed, there is a lack of universal findings concerning the clinical manifestation of the infection; extraluminal parasites were found in biopsy specimens from patients with diarrhea [19,21,22], a patient with asymptomatic giardiasis with steatorrhea [20], and here, a patient with cancer and coincidental asymptomatic giardiasis. The differences in clinical scenarios thus indicate that the invasive potential alone, if any, cannot account for pathological conditions promoting carcinogenesis.

Using a conventional genotyping approach, we further showed that in our case, the infecting *Giardia* belonged to the sub-assemblage AII genetic group, similar to *Giardia* that was found to invade the epithelium during symptomatic giardiasis in a child from Mexico [22]. Although it is notable that both cases were caused by *Giardia* from the same genetic group, these findings are most likely coincidental because the sub-assemblage AII is common and dispersed worldwide [23].

In conclusion, we present a case of coincidental giardiasis and adenocarcinoma in the head of the pancreas and show the presence of parasites situated between and below the epithelial lining of the duodenum. This atypical localization suggests that the coinfecting population of *G. intestinalis* is able to either actively penetrate the epithelium or passively traverse the epithelial barrier, the integrity of which may be disrupted, e.g., in association with inflammation reactions related to cancer.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parint.2019.04.013.

Declaration of interest

The authors have no conflicts of interest to declare.

Ethics statement

Approval for the publication of this case report has been obtained from the Ethics Committee of Faculty Hospital Ostrava (Certificate No. 736/2018). All the procedures in the study involving human material and data were performed in accordance with *World Medical Association's Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects* [24].

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